LXXXI. THE MECHANISM OF DEGRADATION OF STARCH BY AMYLASES

IV. THE ACTION OF MALT AMYLASE ON α -AMYLODEXTRIN

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IN the course of some experiments on the hydrolysis of α -amylodextrin (Baker) (erythrogranulose of Wijsman) by malt amylase, it was observed that the mutarotation exhibited by the fission products was in the α -sense, whereas the same enzyme acts on starch, amylose etc., to yield products which exhibit mutarotation in the β -sense. It seemed that, in α -amylodextrin, we had a substrate which itself determines the sense of mutarotation, producing an α -effect with a predominantly β -amylase. However the subsequent work and conclusions of Freeman & Hopkins [1936, 2] were opposed to the hypothesis that the sense of mutarotation of the fission products was characteristic of the substrate and not of the enzyme. Nevertheless the investigations prosecuted with α -amylodextrin warrant discussion as they serve to bring to notice a number of interesting facts.

Haworth *et al.* [1935] found that a preparation of α -amylodextrin, on acetylation followed by methylation of the acetate, yielded a methylated amylodextrin, analysis of which indicated that α -amylodextrin has a chain length of 16–17 glucose units. There was no evidence to show that, except in the matter of chain length, the amylodextrin molecule was different in structure from that of starch (about 25–30 glucose units). One significant point, however, was that phosphorus was present in the dextrin in more than twice the percentage normal for potato starch and appeared to be chemically combined.

Experiments reported in this communication lead to the conclusion that there is some structural difference, of character quite unknown, between the chain of α -amylodextrin and that of starch. The persistent difficulty experienced in hydrolysing α -amylodextrin by malt amylase, particularly in the later stages, and the high proportion of glucose found in the reaction products of the "difficult hydrolysis" are indications of a feature of molecular structure not present in the remainder of the starch. Other workers using amylases have arrived at such conclusions, e.g. Ling & Nanji [1923]. Admittedly the possibility must be borne in mind that α -amylodextrin may be a synthetic product of enzyme action, but such resynthesis is improbable; it does not occur when amyloamylose is hydrolysed by barley amylase. Further it may be that the phosphorus present has some influence in inhibiting degradations by amylases in certain cases, but the quantity of phosphorus found in α -amylodextrin by Haworth *et al.* (0.48% P_2O_5) is inadequate to provide even one atom per three chemical molecules.

It was found that malt amylase promotes the hydrolysis of α -amylodextrin at a relatively high rate until about 35–40% (as maltose) of the theoretical

hydrolysis is attained, later at a much lower and more nearly linear rate. The point of change is fairly well marked and depends on the activity of enzymes present. During the first (fast) phase the fission products appear to be maltose and reducing dextrins, with very little or no glucose. Mutarotation of these products was in the α -sense and agreed quantitatively with reasonable closeness with the supposition that β -maltose and α -dextrins were formed, the latter presumably by the action of the α -, the former by that of the β -amylase. During the second (slow) phase, apparently only glucose is formed as reducing sugar. The hydrolysis could not be promoted rapidly enough for the sense of the mutarotation to be observed. Although a total hydrolysis equivalent to 96–97% (maltose) was attained, so much of the reducing matter was actually glucose, that an appreciable residue of incompletely hydrolysed matter remained, equivalent to perhaps 30% of the α -amylopectin.

EXPERIMENTAL

α -Amylopectin and malt amylase were prepared as described by Freeman & Hopkins [1936, 1, 2], except that the procedure of fractionation of the latter into the α - and β -components was omitted. The preparation was free from maltase.

For the observations of mutarotation the reaction mixture was poured through a specially prepared Büchner filter passing 20 ml. of liquid in 3–5 min. The filter, which was devised by one of us (K. B.), had the property of adsorbing all suspended material (much of which would pass an ordinary filter) and all, or almost all, of the enzyme. It is also evident, from a consideration of the rotations of the filtrates recorded in Table II in conjunction with the concentrations of amylopectin in the reaction mixtures from which they were respectively obtained, that much of the more complex dextrin had been held back. The technique had also the advantage that enzyme action was proceeding until the liquid passed the filter, and then ceased. As soon as just enough had passed through, a 1 dm. polarimeter tube was filled and a first reading was secured, while 10 ml. of the same filtrate were run into 1 ml. of N Na_2CO_3 to complete the mutarotation. After 20–30 min. the latter was filtered and its rotation was taken. This was found to agree well with the final rotation of the portion previously read, thus indicating that no enzyme action was proceeding after filtration.

Preparation of the filter. A suspension of ferric phosphate was prepared by adding 11% disodium phosphate ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$) to 4% FeCl_3 at room temperature with rapid stirring until the mixture was exactly neutral to litmus. (It is important not to allow the mixture to become alkaline to litmus during mixing, otherwise ferric hydroxide is deposited). The precipitate was washed by decantation until free from chloride and stored in suspension. A large Büchner funnel, adapted to a small receiving flask, was fitted with a damp filter and the ferric phosphate poured on to form a fine skin on the surface of the paper. The film was sucked free from excess of water but not allowed to dry. Other adsorptive materials had been tried, e.g. forms of aluminium, ferric and chromium hydroxides, but were less satisfactory.

Exp. 1. Kinetics of hydrolysis of α -amylopectin by malt amylase.

1.0 g. of α -amylopectin + malt amylase as indicated in Table I. Total reaction vol. 15 ml., temperature 22°, pH 4.6, acetate buffers 0.06 N . 2 ml. withdrawn at times stated, run into iodine and titrated iodimetrically. The results are expressed as percentage of the maltose theoretically obtainable at complete hydrolysis.

Table I

Malt equivalent of amylase g.	% hydrolysis					
	7 min.	1 hr.	3 hr.	6 hr.	24 hr.	48 hr.
1	5.8	21.2	29.1	33.4	38.4	42.2
2	9.9	26.2	32.5	36.4	43.0	47.3
4	16.4	30.3	36.3	39.7	46.9	54.9
8	20.8	33.0	39.1	42.5	54.8	67.9
16	24.7	36.8	41.0	45.1	62.6	78.0

Exp. 2. The sense of mutarotation of reaction products.

Weights of α -amylodextrin + malt amylase equivalent to weights of malt and total volumes as stated in Table II. Temperature 18°, pH 4.6 throughout, 0.02 *N* acetate.

Table II

Malt equivalent of amylase g.	α -Amylo-dextrin g.	Total vol. ml.	Period of filtration min.	Approx. reducing power of filtrates, maltose %	Time of reading min.	Angular degrees (1 dm.)		
						Rotation	Rotation after alkali (corrected)	Mutarotation
62.5	4.6	48	8-15	1	17.0	9.38	9.27	-0.11
			22-26	2	27.0	14.57	14.55	-0.02
120	4.6	60	6-10	2	10.5	8.87	8.82	-0.05
			12-18	3.3	20.0	12.37	12.32	-0.05
			12-28	3.5	29.0	12.73	12.59	-0.14
100	6.54	80	14-20	2.9	21.0	11.80	11.68	-0.12
			30-32	2.6	33.0	9.76	9.76	0.0
			30-45	3.4	47.0	12.48	12.35	-0.13
100	7.3	80	7-10	2	11.3	10.11	10.04	-0.07
			10-22	2	23	8.79	8.74	-0.05
200	20.0	100	7-13	1.5	15	8.90	8.92	+0.02
			22-34	2	36	9.43	9.36	-0.07
			22-67	5.4	69	25.28	25.27	-0.01

The same preparation of malt amylase, under conditions parallel with those of Exp. 2 but with soluble starch as substrate, gave decided β -mutarotations, e.g. +0.36° and +0.40° being observed.

The combined filtrates from the last hydrolysis of Exp. 2 (Table II) were fractionated as described in Exp. 3. The fraction extracted by absolute alcohol contained both maltose and glucose. The osazones were isolated and recrystallized; the glucosazone had M.P. 207°, and on admixture with authentic glucosazone, M.P. 210°, the mixture had M.P. 208°.

Exp. 3. The sugars formed in the early stages of hydrolysis.

Reaction mixtures were prepared containing weights of α -amylodextrin of moisture-free content stated in Table III together with malt amylase equivalent to 22.5 g. of malt and sufficient acetic acid to bring the mixture to pH 4.6. Acetate buffer was added, 0.01 *N* in the final reaction mixture of 500 ml. The courses of the reactions at 25° were followed and at the times stated in the table the reactions were checked and treated exactly as described by Freeman & Hopkins [1936, 1]. The sugars extracted by 95 % alcohol were determined by their optical rotatory powers and by iodimetric titration. From these results the weights of maltose and glucose were calculated.

Table III. *Analysis of products of hydrolysis of α -amylodextrin by malt amylase*

	A	B
Weight of moisture-free α -amylodextrin, g.	9.54	9.035
Apparent maltose in reaction mixture as percentage of theoretical:		
After 1 hour	40.4	36.5
After 1.5 hours	43.6	39.9
After checking reaction	48.1	
Apparent weight of maltose in portion fractionated, g.	4.30	3.72
Containing maltose, g.	3.60	3.02
glucose, g.	0.00	0.00
Osazone	Maltosazone	
Dextrin in portion fractionated, reducing power as maltose, g.	0.70	0.70

Exp. 4. The sugars formed in the late stage of hydrolysis.

The dextrins recovered from Exp. 3 (A and B) were dried in a Fischer drier at 70° for 2 days. Portions as stated in Table IV were weighed out and treated with malt amylase (quantities as indicated) and the procedure of Exp. 3 repeated.

Table IV. *Analysis of products of hydrolysis by malt amylase of the residual dextrins from Table III*

Weight of residual (moisture-free) dextrin, g.	3.698	2.262
Weight of malt amylase, g.	0.136 ($\equiv$ 10 g. malt)	0.544 ($\equiv$ 40 g. malt)
Apparent maltose in reaction mixture as percentage of theoretical:		
After 24 hours	55.05	84.98
After 42 hours	—	104.8
After 44.5 hours	61.09	—
(More amylase now added)	($\equiv$ 20 g. malt)	
After 70 hours	78.6	—
After 94 hours	87.95	—
Weight of reducing sugar in portion fractionated, as glucose, g.	1.37	1.193
Comprising glucose, g.	1.168	1.190
maltose, g.	0.146	0.014

DISCUSSION

The mutarotations observed in Exp. 2 were small and almost invariably in the α -sense. The magnitudes were quite inadequate to be accounted for as α -maltose. Experiment with a 1.9% solution of β -maltose, freshly prepared at 20° and pH 4.6 (0.02 *N* acetate content), indicated that mutarotation up to +0.30° (1 dm.) could be measured subsequently to the first 10 min. The conditions of time, temperature and pH and acetate buffers were chosen to resemble those obtaining in some of the filtrates described in Exp. 2 (Table II), which contained reducing matters equivalent to 2% of maltose undergoing mutarotation. The inference is drawn that if β -maltose had been the sole reducing product in these filtrates, mutarotations of approximately +0.30° would have been observed. α -Maltose, or dextrins of equivalent mutarotating power, would correspondingly have yielded about -0.45° of mutarotation, assuming the value of $[\alpha]_D + 168^\circ$ for α -maltose. The mutarotations observed in the actual filtrates in question were however about -0.05° and -0.07°, approximately the arithmetic mean of the above values. It is inferred that on the one hand the α -amylase produced α -dextrins and α -maltose, and on the other hand β -amylase

produced β -maltose, and at approximately equal rates. No glucose was formed at this stage of the hydrolysis, as was indicated by analyses (not recorded) of some of the filtrates.

Notwithstanding that the β -amylase greatly preponderates in malt amylase, which gives β -mutarotating fission products with starch (as was confirmed with the preparation used in this work), the products of its action on α -amylodextrin appear, in the early stages, to be equivalent proportions of α - and β -products. This suggests that for each α -reducing group exposed or formed by the α -amylase, one β -reducing group is exposed or formed by the β -amylase. The excess of the latter enzyme should lead to a preponderance of β -maltose, but such is not the case. Possibly, for each break in the dextrin molecule effected by the α -amylase, only one maltose molecule can be removed before a change in the structure imposes a fresh barrier to the β -enzyme, or the removal suffices to degrade the fragment to maltose. The possibility also exists that the maltose is a secondary product due to synthetic action of an enzyme present.

Exps. 3 and 4 show that only maltose (and reducing dextrins) is formed in the first phase of the reaction, corresponding to the rapid reaction (Table I) up to about 40% hydrolysis, but that glucose formation is characteristic of the second phase, 40–90% (as maltose). The increasing formation of glucose largely accounts for the apparent comparatively linear character of the reaction velocity during this phase, the glucose having a higher reducing value than maltose. The mutarotation of the fission products formed during this second phase could not be measured.

SUMMARY

α -Amylodextrin at normal temperature is degraded by malt amylase in two fairly well defined stages.

(1) A relatively rapid reaction in which maltose is the principal sugar produced, but in the mutarotation exhibited, the α -effect faintly predominates. Probably α -dextrins and β -maltose are formed in equivalent reducing (and mutarotating) proportions.

(2) A relatively slow reaction, apparently linear, during which glucose is the chief if not the only reducing sugar formed, thus accounting for the observed linearity. The mutarotation of the fission products at this stage could not be measured.

There is no well defined point of demarcation between these stages of the reaction unless low concentrations of the enzyme are employed.

A new technique for determining the sense of mutarotation in enzyme-substrate mixtures of highly colloidal character is described.

REFERENCES

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